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结瘤因子的研究进展*

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摘要：结瘤因子是由根瘤菌产生的一类信号分子，它们在结瘤的起始阶段发挥着十分重要的作用。新近的研究结果证明结瘤因子大分子骨架上的不同侧链基团是决定细菌与宿主植物间相互识别的关键因素，根瘤菌细胞中一系列结瘤基因编码能够合成 Lipo-chito-oligosaccharides（LCOs）的各种酶类，进而确定结瘤信号分子的特定结构。目前，一系列令人兴奋的实验结果表明：LCOs 不仅可促进豆科作物的生物固氮作用，对一些非豆科作物的细胞分裂作用等同样具有刺激作用。对根瘤菌结瘤因子的研究显然有助于进一步了解细菌与植物的相互作用机理，并进而为农业生产带来直接利益。本文在综述这方面的研究进展同时，还就瘤菌、豆科作物和结瘤信号分子之间的相互作用机理，以及根际促生细菌、水杨酸和结瘤信号分子之间的可能关系进行了理论分析。

关键词：结瘤因子；结瘤作用；根瘤菌；豆科作物

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Nod Factors（Lipo-Chito-Oligosaccharides）Research Progress

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Abstract：Nod factors are a novel general class of signal molecules produced by rhizobia，which play a key function in the initial steps of nodulation. Research has shown that different substitutions on a lipo-chito-oligosacchride（LCO）backbone play a role in deciding the specificity between bacteria and the host plants. The nod genes of rhizobia，which contain the genetic basis for this structural variety，include a set of nodulation genes encoding the enzymes that synthesize LCOs. There are promising results that suggest the application of LCOs in leguminous and non-leguminous plants can control cell division. Research in this area will pave the way for fully understanding the interactions between bacteria and plants，and eventual ap-

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plication of this information for agronomic benefit.

Key words: Nod factors; Nodulation; Rhizobacteria; Leguminous plants

1 Introduction

Lipo-chito-oligosaccharides (LCOs) are a general class of signal molecules secreted by rhizobia, in response to isoflavonoid and flavonoid signals from their specific hosts. This kind of signaling was discovered as a result of studying root nodulation processes in leguminous plants (Spaink, 1996). Since all of these molecules were found to be acylated forms of small chitin fragments, they have been called lipo-chito-oligosaccharides. From the time Lerouge *et al*. (1990) reported the purified active compound using *Sinorhizobium meliloti* culture filtrates and identified the chemical structure as a substituted lipo-chitin molecule, this research area has attracted much interest from researchers in biochemistry, genetics, microbiology and agriculture. Rhizobia elicit the formation of new organs from their specific leguminous hosts, called nodules, which are the sites of the N_2-fixing symbiosis, and their establishment requires coordination of activities between rhizobia and many leguminous plus a few of non-leguminous plants. Rhizobia are now classified into four genera: *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium* and *Azorhizobium*, collectively referred to as rhizobia. The *Leguminosae* is a large family containing more than 15, 000 species, most of which can be nodulated by rhizobia, and these nitrogen-fixing associations have considerable ecological and agronomic importance (Denarie *et al*, 1996). In this review, the authors sum up some recent research results concerning the genetics, molecular structure and functions of LCOs, and analyze the underlying relationships with other beneficial microbes and salicylic acid (SA).

2 Function of Signaling in the Process of Nodule Formation and the Structure of LCOs

Nodule development and subsequent nitrogen fixation is a complex process involving a large number of genes. They are often conserved, closely associated, and in some cases, on a single plasmid (Paul & Clark, 1990). In most rhizobia, one nodulation gene (nodD) is constitutively expressed, while the others are expressed only in the presence of host plants or their flavonoid extracts, such as luteolin or genistein. Gagnon & Ibrahim (1998) reported a novel family of nod gene inducers of some rhizobia. The nodulation process begins with reproduction of bacteria in the rhizosphere, followed by chemotaxis toward plant exudates, adhesion to the root and infection. Among those processes, mutual exchange and recognition of signal molecules between bacteria and plant roots are very important in the first steps of nodulation, which can be briefly summarized as follows:

(1) Growth and reproduction of rhizobia in the rhizosphere, their movement and attachment to the plant roots under suitable soil physiochemical conditions, which involve chemotaxis, electrotaxis, and moving by water flow.

(2) Flavonoids (and possibly other signals) excreted by plant roots.

(3) Plant signals are recognized by bacterial *nod*D gene products (NodD) leading to the induction of the biosynthetic pathway involved in nodulation.

(4) Secretion of lipo-polysaccharide signals from bacteria determine the specific host. Different rhizobia secret different signals, which determine specific recognition between bacteria and plant host.

(5) Combined signaling and specific receptors on the root surface, which may also become associated with a secreted bacterial protein (recadhesin) and plant-encoded lectins, interact with the bacterial polysaccharides.

(6) Infection of root hairs, root hair curling, deformation and infection thread formation occur in many agricultural legumes.

(7) Finally, the plant early and then late nodulin genes are expressed, bacternoids are eventually formed in the nodules and N_2-fixation begins.

From the description above, it can be seen that nodulation is a multiple step process that involves interactions between specific plants and bacteria, in which LCO signaling is one of the most important causative agents.

The rhizobia-legume symbiosis is highly specific. The bacterial Nod factor signals (NFs) are major specificity determinants that trigger the nodulation program in a compatible host. At extremely low concentrations, purified NFs are capable of eliciting, in an homologous legume host, many of the characteristic plant development responses, including cell divisions, and the triggering of a plant organogenetic program. The type of infection, as well as the nodule structural and development characteristics, are specified by the plant and not by the rhizobial strain, indicating that the host possesses the genetic information for symbiotic infection and nodulation and that the role of the bacteria is to turn on this program with specific signals (Denarie *et al*, 1996).

Nod factors are lipo-chito-oligosaccarides varying in oligosaccharide chain length, the nature of the fatty acids and substitutions on the oligosaccharide (Mergaert, *et al*, 1997). Different combinations of substituents in LCOs have been reported (Stokkermans *et al*, 1996; Folch-Mallol *et al*, 1996). Substitutions on the oligosaccharide backbone structure can play a role in determining the host range of the rhizobia or might play a general role in the capacity of LCOs to induce plant morphogenesis. The present knowledge of the biosynthesis of LCOs has been an additional basis for assigning biological functions to various substitutes. Schlaman *et al* (1997) used ballistic microtargeting as a novel approach to deliver derivatives of the nodulation signal molecules inside the roots of *Vicia sativa*. The results show that O-acetylated chitin oligosaccharides can induce root cortical cell divisions when delivered by microtargeting, and the data favour a model in which the oligosaccharide moiety of the rhizobia LCOs induces cortical cell division and the fatty acyl moiety plays a role in transport of LCOs into the plant tissue. Lopez-Lara *et al*. (1995) reported that an N-vaccenoyl-chitopentaose bearing an N-methyl group is able to induce nodule primordia on *Phaseolus vulgaris*, *Acacia cyanophylla*, and *A. melanoxylon*, indicating that for these plants an N-methyl modification is sufficient for nodule primodia induction. Nodulation studies show that *Lotus* plants are nodulated by *R. loti*, but not by most other *Rhizobium* strains, indicating that *R. loti* produces specific LCOs which are necessary for the nodulation of *Lotus* plants. The LCOs produced by five different *R. loti* strains have been purified and were showed to be N-actylglucosamine pentasaccharides of which the non-reducing residue is N-methyl-

ated and N-acylated with cis-vaccenic acid (18:1) or stearic acid (18:0) and carries a carbamoyl group (Lopez-Lara *et al*, 1995). *R. fredii* HH103 produces a large variety of nodulation factors consisting of a linear backbone of GlcNAc with different degrees of polymerization, bearing on the non-reducing residue various different N-acyl residues. The reducing terminal residue is 2-O-methylfucosylated at position 6 (Gilserrano *et al*, 1997). By the use of the wild-type and mutants of *Azorhizobium caulinodans* ORS571, the role of the different Nod factor glycosylations on nodulation behavior was investigated. The mere presence of the D-arabinosyl group at the reducing end of the LCOs resulted in a higher number of nodules on roots of *Sesbania rostrata*, whereas the presence or absence of L-fucose had no effect. The situation is just the opposite in other tropical legumes (Fernandezlopez *et al*, 1998).

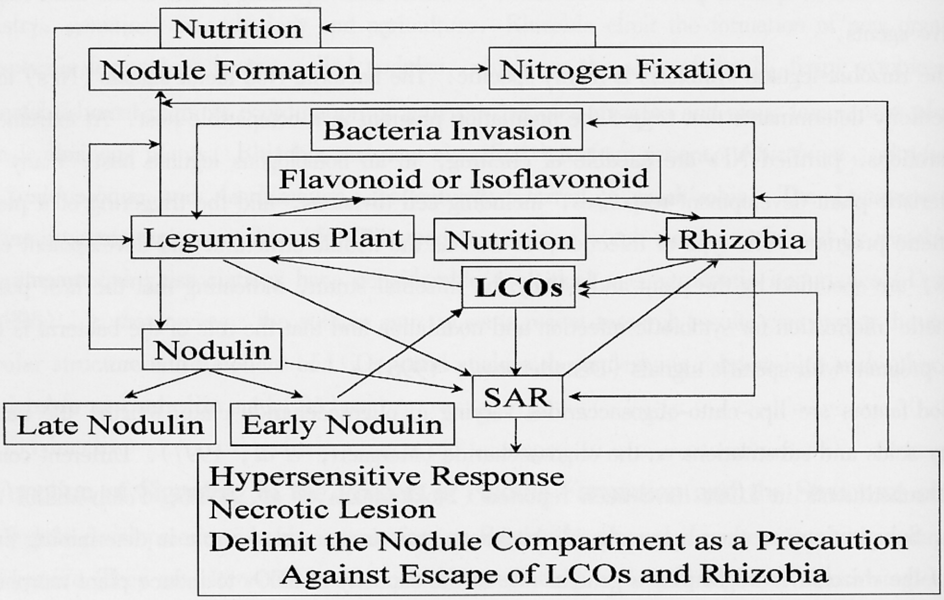

Fig. 1 The Possible interaction among microbe, leguminous plant and LCOs.

Chitin oligosaccharides released from fungal pathogens induce plant defense reactions in rice, while LCOs induce the development of a new plant organ, the nodule, in legumes during infection by rhizobia. The former situation is pathogenic and the latter situation beneficial to the plant. In its broadest sense, the term plant parasite indicates any microorganism that lives together with plants and uses plants compounds as nutrients. A parasite interaction can be symbiotic when the microorganisms return something to the pant or pathogenic when it harms the plant. With the framework of this definition, it is easy to see that pathogenesis and symbiosis can have common aspects, especially those of plant control over invasion. Rhizobia use a form of chitin signaling to trigger plant responses for nodule formation. It is not surprising that the plant to developes chitinase in a feedback control for inactivation of the signal. Indeed, excessive amounts of mitogenic Nod factors could disturb plant growth or lead

to elicitation of plant defense responses that might disturb the interaction.

Both pathogens and symbionts must have developed ways to avoid, suppress, or overcome defense responses of the host, and then eventually elicit them. Furthermore, in both types of interactions, the host responds by deploying sets of similar tools, for instance, chitinase, plays a role in autoregulation of nodulation (Sofie, 1998). Figure 1 shows the interaction between microbes and leguminous plant.

Stacey and Shibuya (1997) compared the plant perception of chitin oligosaccharides between legumes and rice. The data support the idea that legumes may possess a second chitin binding receptor that shows a greater specificity for the LCOs signals. The presence of this second receptor may be one of the key factors that distinguishes plants capable of nodulation by rhizobia (e. g., soybean) from those that cannot be nodulated (e. g., rice).

Clearly, the effect of LCOs in the non-leguminous plants is interesting. Preparations of Nod factors from various bacteria, highly purified LCOs from *Rhizobium* sp. NGR (pA28) induced alkalinization in the tomato cell cultures at nanomolar concentrations. This demonstrates that LCOs can be perceived by tomoto, a nonhost of rhizobia (Staehelin *et al*, 1994). LCOs may be able to initiate nodule growth in non-legumes as well. Thus, they may also serve as general-purpose plant growth regulators (Roehrig *et al*, 1995). Stokkermans *et al*. (1995) demonstrated that synthetic analogs of natural product Nod factors have the same biological activities. The object of these experiments were to determine structure-activity relationships, a collection of synthetic and natural product LCOs was assayed on *Glycine soja* which induced both root hair deformation and nodule initiations.

LCOs are probably novel representatives of a general class of signal molecules involved in plant morphgenesis. The results above give us beneficial clues as to whether natural or artificial LCOs may have analogous functions for plants and the possibility to cross the first host range barrier originally depending on the mutual recognition of signals that are exchanged to form more effective nitrogen fixation symbioses in nature. The utilization of LCOs in non-leguminous plants to promote plant growth may also be hopeful and promising.

3 The Genes of LCO Synthesis

The nod genotype of rhizobia, which forms the genetic basis for the structural variety of Nod LCOs, includes a set of nodulation genes encoding the enzymes that synthesize LCOs. Currently, more than 50 nodulation genes have been identified in different rhizobia. Every strain has its own specific set that defines the nod genotype (Mariangela & Gary, 1997). Nodulation genes are included the following three general catagories:

(1) The common nodulation genes (*nodABC*), present in all rhizobia species, which are induced by host signal molecules. A single 10 Kb region on the sym plasmid can activate all of the plant nodulin genes and result in nodule formation.

(2) The regulatory gene (s) (such as nodD) that positively regulate nod gene transcription. In *Bradyrhizobium*, the nodD gene is constitutively expressed. Only after the nodD gene product com-

bines with inducers can common nod gene transcription begin. *nod DABC* (and probably just ABC) are required to initiate the early events in the symbiosis. Some strains have more than one regulatory gene, such as nolA and *nodVW* in *B. japonicum*. Folch-Mallol *et al*. (1998) conducted experiments suggesting that the *nodHPQ* genes are organized as an operon that is transcribed in a *nodD*-independent manner and is not regulated by flavonoids.

(3) Structural genes, which include genes that determine host specific nodulation (*hsn*) and genotype specific nodulation (*gsn*) genes, appear to specify the ability to nodulate selected genotypes within a legume species (Mariangela & Gary, 1997). Most of the nodulation genes belong to this class. In addition, fix genes which are involved in the process of nitrogen fixation, *nif* genes which are a subset of fix genes and code for nitrogenase and related proteins, *ntr* genes which code for the enzymes of nitrogen assimilation, *exo* and *ndv* genes which are involved in the production of acidic exopolysaccharides on the surface of *S. meliloti* are also categorized in this group.

In recent years, a number of new *nod/nol/noe* genes, with new functions, have been discovered and some of the boundaries of the nodulation gene category have become less clear. Here we summarize some of the main recent findings.

The common *nodABC* genes, present in all rhizobia, are required for the synthesis of the core structure of Nod factors. *nodC* is an N-acetylglucosaminyltransferase; and *nodB* is a chitooligosaccharide deacetylase; *nodA* is involved in N-acylation of the amino-sugar backbone. Specific *nod* genes are involved in diverse Nod factor substitutions that confer plant specificity. Studies of Nod factors from *nodA*, *nodB*, *nodC*, and *nodI* mutants indicate that (1) *nodA* of *S. meliloti*, in contrast to *nodA* of *R. tropici*, is able to transfer unsaturated C16 fatty acids onto the chitin backbone and (2) *nodC* of *R. meliloti* specifies the synthesis of chitin tetramers. These results show that allelic variation of the common *nodABC* genes is a genetic machanism that plays an important role in signal variation and in the control of host range (Roche *et al*, 1996).

In *R. leguminosarum*, the *nodABC* and *nodFEL* operons are involved in the production of lipochitin oligosaccharide signals that mediate host specificity. Of the *nod* genes, induction of only *nodFE* is sufficient to modify fatty acid biosynthesis to yield trans-2, trans-4, trans-6, cis-11-octadecatetraenoic acid, with an absorbance maximum of 303 nm. This unusual C18:4 fatty acid is not only found in the lipo-chito-oligosacchrides but is also associated with the phospholipids. Other *nodFE*-derived fatty acids, a C18:3 trans-4, trans-6, cis-11-octadecatrienoic acid that has a characteristic absorption maximum at 225 nm, and a C18:2 octadecadienoic acid, were also found (Geiger *et al*, 1998). Using *R. tropici* CIAT899 strain, a novel LCO consisting of a linear backbone of 4 N-acetylglucosamine residues and one mannose that forms the reducing-terminal residue and bearing a C18:1 fatty acyl moiety on the non-reducing terminal residue were described. In addition, the authors identified, cloned, and sequenced the *nodH* and *nodPQ* genes, generated mutations in the *nodH* and *nodQ* genes, and tested the mutant strains for nodulation in *Phaseolus* and *Leucaena* plants. The results indicate that the sulfate group present in wild-type Nod factors plays a major role in nodulation of *Leucaena* plants (Folch-Mallol *et al*, 1996).

The LCOs synthesized by *R. leguminosarum* bv. *trifolii* were analyzed using positive mode fast atom bombardment and positive and negative mode electrospray ionization mass spectrometry (Van Der Drift *et al*, 1996). The results show that the bacterial strain investigated produces *nodE*-dependent LCOs with highly unsaturated fatty acyl moieties, and the *nodE* gene determines the host specificity of the strain.

Bloemberg *et al*. (1995) proposed that the nodulation host range of the *R. leguminosarum* biovars *viciae* and *trifolii* is determined by the degree of hydrophobicity of the polyunsaturated fatty acyl moieties of their LCOs, which is mediated by the host-specific central domain of the *NodE* protein. The findings of Hanin *et al*, (1997) showed that *noeE* is a host-specificity gene which probably encodes a fucose-specific sulphotransferase.

The products of the bacterial *nodIJ* genes are related to transporters of capsular polysaccarides and were proposed to be involved in LCO transport. In *E. coli* as well as in *Azorhizobium*, the *nodIJ*-encoded transporter showed a specificity for more hydrophilic LCOs (Fernandezlopez *et al*, 1996). A kinetic analysis of secretion of lipo-chito-oligosaccharides produced by an *R. etli* wild-type strain and derivatives carrying disrupted *nodI* or *nodJ* genes was performed. In contrast, strains carrying *nodI* or *nodJ* mutations secreted less LCO, and accumulated LCO metabolites intracellularly after 4h of induction (Cardenas *et al*, 1996).

Bloemberg *et al*. (1995) analyzed the enzymatic properties and substrate specificity of the *NodL* protein. Their results indicated that the nonreducing terminally de-N-acetylated chitin oligosaccharides produced by the *NodC* and *NodB* enzymes are the in vivo acetyl-accepting substrates for the *NodL* protein.

Several lines of evidence indicate that the *nodS* gene located in the *nodABCSUIJ* operon is implicated in the methylation of Nod factors. Geelen *et al*. (1995) presented an *in vitro* assay showing that NodS from either *A. caulinodans* or *Rhizobium* species NGR234 methylates end-deacetylated chitooligosacchrides, using [3H-methyl] -SAM as a methyl donor.

R. leguminousarum bv *viciae* strains produce LCOs that are O-acetylated at the reducing terminus and are required for nodulation of wild pea cultivars originating from Afghanistan that posses the recessive *sym*2 (A) allele. The O-acetylation of the reducing sugar of LCOs is mediated by the bacterial nod gene, which presumably encodes an acetyltransferase. For nodulation on Afghan pea cultivars and *sym*2 (A) introgression lines the *nodX* gene can be functionally replaced by the *nodZ* gene of *B.-japonicum*, which encodes a fucosyltransferase that fucosylates the reducing terminus of LCOs (Ovtsyna *et al*, 1998).

A mutation in *nodZ* of *B. japonicum* results in the synthesis of nodulation signals lacking the wild-type 2-O-methylfucose residue at the reducing-terminal N-acetylglucosamine. Lopez-Lara *et al*. (1996) reported that transfer of *nodZ* to *R. leguminosarum* biovar. viciae, which produces LCOs that are not modified at the reducing-terminal N-acetylglucosamine, results in production of LCOs with a fucosyl residue on C – 6 of the reducing-terminal N-acetylglucosamine. This finding, together with *in vitro* enzymatic assays, indicates that the product of *nodZ* functions as a fucosyltransferase.

It is apparent that knowledge of LCO genes is very important, not only for understanding the mechanism of LCO function but also in the application of LCOs.

4 Possible Relationship Among LCOs, PGPR and Salicylic Acid — An Hypothesis

Plant growth promoting rhizobacteria (PGPR), when applied to seeds, tubers or roots, have been shown to colonize plant roots and increase plant yields. The mechanism (s) by which PGPR increase plant growth are not well understood but production of plant hormones or other metabolism products, enhancement of plant nutrient uptake or suppression of pathogenic or other deleterious organisms via antibiotic or siderophore products have all been postulated (Saxena & Tilak, 1994). For example, preincubation of *B. japonicum* with a strain of *Pseudomonas fluorescence* further increased the level of nodulation indicating a bacteria-bacteria interaction (Botton *et al*, 1990). Smith and colleagues (Zhang *et al*, 1996; 1997; Dashti *et al*, 1997; 1998) have proven that soybean seedling growth can be promoted at low root zone temperatures using two PGPR strains of *Serratia* that produce plant growth promoting compounds. Sometimes, PGPR strains have no effects on plant growth. This may be related to certain environmental conditions. Surange & Kumar (1993) and Halder & Chakrabartty (1993) reported that rhizobia possess the potential to solubilize soil phosphorus which suggests that rhizobia can promote plant nutrient uptake when in the rhizosphere. More recently, rhizosphere colonization and growth promotion of non-legumes by *Rhizobium leguminosarum* biovar *trifolii* were reported (Hoflich *et al*, 1995; Yanni *et al*, 1995). Chabot *et al*. (1996) suggested that the mechanisms of action of growth promotion of non-legumes by rhizobia may be similar to other PGPR of non-legumes and should be investigated further. Some kinds of PGPR can form exo-polysaccharides similar to those produced by *Rhizobium*, under certain cultural conditions. It has been reported that the complex formed by polysaccharides and proteins possesses important significance in recognition, transport, protection, adhesion and lubrication (Voet *et al*, 1995). Recent observations of D'Haeze *et al*. (1998) suggest that Nod factors are necessary for entry in the process of nodulation and surface polysaccharides of rhizobacteria are important in subsequent events.

SA has been thought to play a key role in the development of systemic acquired resistance (SAR). This hypothesis was based on the observation that the endogenous levels of SA increase locally and systemically in tobacco plants inoculated locally with tobacco mosaic virus (Malamy *et al*, 1990). SA also increases in the phloem of infected cucumber before the expression of SAR, consistent with a role as a signal for SAR (Metraux *et al*, 1990; Rasmussen *et al*. 1991). Furthermore, SA was reported to increase pod number and yield in mung bean (*Vigna radiata* L. Wiliczek) (Kling and Meyer, 1983; Lian *et al*. 2000). Jain and Srivastava (1981) found that SA increased the *in vivo* activity of nitrate reductase in corn seedlings. Zhou *et al*. (1999) demonstrated that the injection of SA into corn can increase photosynthetic rates by 42% when compared with distilled water, and corn plants injected with SA produced 9% more grain yield than plants injected with no plant growth regulators. Lian *et al*. (2000) found that 5 mM SA had negative effects on soybean seedling development, while 0.1 mM SA can promote soybean seedling growth. In addition, soybean seedling growth in sterile soil

was reduced due to repressed nitrogen uptake following addition of 5 mM SA, indicating that some concentrations of SA can alter the N nutrition of seedlings. For these reasons SA was postulated as a regulator of photosynthetic homeostasis.

It is known that genistein is the plant-to-bacteria signal most effective in inducing LCO secretion by *B. japonicum*. We can use this as a way to induce LCO formation and increase soybean nodulation. Zhang & Smith (1995) and Zhang *et al*. (1996) conducted experiments showing that adding genistein increased soybean nodulation and nitrogen fixation in growth chamber studies, and genistein-preincubated *B. japonicum* increased the grain yield and protein yield of soybean. In fact, the positive effects of these experiments are because of the formation of LCOs by *B. japonicum*. LCOs possess beneficial functions for plants in the following ways: deformation of root hairs, induction of nodulation genes, deformation and branching of roots, stimulation of cytoplasmic streaming, depolarization of membrane potential, formation of nodule primodia in the root cortex and preinfection thread induction of flavonoid synthesis genes (Spaink, 1996).

Bringing all of this together, we postulate the existence of some underlying mechanism common to LCOs, PGPR and SA that stimulates plant growth. Figure 2 shows possible relationships among them.

Fig. 2　Probable relationship among PGPR, LCOs and SA in stimulation plant growth or increased nodulation. Filled arrows illustrate portions ofthe pathways proposed from direct evidence. Broken arrows illustrate inferred pathways or interactions.

5　Conclusions

Specificity in plant-rhizobia interactions is likely to be determined by recognition steps involving LCOs, their inducers and receptors. LCOs are the prerequisite for functioning not only in recognition between plant and bacteria, but also in the bacterial ingress that leads to nodulation. Various LCOs give rise to nodulation in different plants, and different bacterial genotypes supply the genetic basis for synthesis of a large variety of LCOs. Several intriguing questions are: What is the pathway for a rhizobia strain secreting LCOs specific to its compatible legumes? Why do LCOs have so many biological functions in some plants? Which kinds of LCOs are the most effective in specific important plants?

What are the location and structure or other features of receptors of LCOs in plant root cells？In this paper we postulate a possible relationship among LCOs，PGPR and SA，through some analogus functions，but it is apparent we lack direct evidence to support this. Of course，further understanding of these questions will provide us with more knowledge about the interactions between plants and microbes. In order to solve the problem，better understand the processes of biological nitrogen fixation，and apply this understanding to non-legumenous plants efficiently，humankind has been walking a long road. It now seems that research bearing directly on these questions is underway.

References：

Bloemberg GV，Kamst E，Harteveld M，*et al*. 1995. A central domain of *Rhizobium* NodE protein mediates host specificity by determining the hydrophobicity of fatty acyl moieties of nodulation factors ［J］. *Molecular Microbiology*，**16** (6)：1123—1136

Bloemberg Guido V，Lagas Ron M，van Leeuwen Steven，1995. Substrate specificity and kinetic studies of nodulation protein NodL of *Rhizobium leguminosarum* ［J］. *Biochemistry*，**34**：12712—12720

Botton H，Turco LF，Kennedy AC. 1990. Rhizoplane colonization of per seedlings by *Rhizobacterium leguminosarum* and a deleterious root colonizing *Pseudomonas* sp.，and effects on plant growth ［J］. *Plant Soil*，**123**：121—124

Cardenas L，Dominguez J，Santana O，Quinto C，1996. The role of the nodI and nodJ genes in the transport of Nod metabolites in *Rhizobium etli*. Gene (Amsterdam)，**173** (2)：183—187

Chabot R，Antoun H，Kloepper JW，*et al*. 1996. Root colonizatio n of maize and lettuce by bioluminescent *Rhizobium leguminosarum* biovar. *phaseoli* ［J］. *Applied and Environmental Microbiology*，**62** (8)：2767—2772

Dashti N，Feng Z，Hynes R，Smith DL，1997. Application of plant growth-promoting rhizobacteria to soybean increases protein and dry matter yield under short-season conditions ［J］. *Plant and Soil*，**188**：33—41

Dashti N，Feng Z，Hynes R，*et al*，1998. Plant growth promoting rhizobacteria accelerate nodulation and increase nitrogen fixation activity by field grown soybean under short season conditions ［J］. *Plant and Soil*，**200**：205—213

Denarie J，Debelle F，Prome JC，1996. *Rhizobium* lipo-chitooligosaccharide nodulation factors：Signaling molecules mediating recognition and morphogenesis ［J］. *Annu Rev Biochem*，**65**：503—535

D'Haeze W，Gao M，Rycke RD，*et al*，1998. Roles for Azorhizobial Nod factors and surface polysaccharides in intercellular invasion and Nodule penetration，respectively ［J］. *Molecular Plant-Microbe Interactions*，**11** (10)：999—1008

Fernandezlopez M，Dhaeze W，Vanmontagu M，*et al*，1998. Changes in the glycosylation pattern at the reducing end of Azorhizobial nod factors affect nodulation efficiency. *FEMS Microbiology Letters*，**158** (2)：237—242

Fernandezlopez M，Dhaeze W，Mergaert P，*et al*，1996. Role of nodI and nodJ in lipo-chitooligosaccharide secretion in *Azorhizobium caulinodans* and *Escherichia coli* ［J］. *Molecular Microbiology*，**20** (5)：993—1000

Folch-Mallol JL，Manyani H，Marroqui S，*et al*，1998. Sulfation of Nod factors via nodHPQ is nodD independent in *Rhizobium tropici* CIAT899 ［J］. *Molecular Plant-Microbe Interactions*，**11** (10)：979—987

Folch-Mallol JL，Marrogui S，Sousa C，*et al*，1996. Characterization of *Rhizobium tropici* CIAT899 nodulation factors：the role of nodH and nodPQ genes in their sulfation ［M］. *Molecular Plant-Microbe Interactions*，**9** (3)：151—163

Gagnon H，Ibrahim RL. 1998. Aldonic acids：a novel family of nod gene inducers of *Mesorhizobium loti*，*Rhizobium lupini*，and *Sinorhizobium meliloti*. *Molecular Plant-Microbe Interactions*，**11** (10)：988—998

Geelen D，Leyman B，Mergaert P，*et al*，1995. NodS is an S-adenosyl-L-methionine-dependent methyltransferase that methylates chitooligosaccharides decetylated at the non-reducing end ［J］. *Molecular Microbiology*，**17** (2)：387—397

Geiger O，Glushka J，Lugtenberg BJJ，*et al*，1998. NodFE-dependent fatty acids that lack an alpha-beta unsaturation are subject to differential transfer，leading to a novel phospholipids ［J］. *Molecular Plant-Microbe Interactions*，**11** (1)：33—44

Gilserrano AM，Francorodriguez G，Tejeromateo P，*et al*，1997. Structural determination of the lipo-chitin oligosaccharide nodulation

signals produced by *Rhizobium ferdii* HH103 [J]. *Carbohydrate Research*, **303** (4): 435—443

Halder AK, Chakrabartty PK, 1993. Solubilization of inorganic phosphate by *Rhizobium* [J]. *Folia Microbiol*, **38**: 325—330

Hanin M, Jabbouri S, Quesada Vincens D, *et al*, 1997. Sulphation of *Rhizobium* sp. NGR234 Nod factors is dependent on noeE, a new host-specificity gene [J]. *Molecular Microbiology*, **24** (6): 1119—1129

Hoflich G, Wiehe W, Hecht-Buchholz C, 1995. Rhizosphere colonization of different crops with growth promoting *pseudomonas* and *Rhizobium* bacteria [J]. *Microbiol Res*, **150**: 139—147

Jain A, Srivastava HS, 1981. Effect of salicylic acid on nitrate reductase activity in maize seedling [J]. *Physiologia Plantarum*, **51**: 339—342

Kling GJ, Meyer MM, 1983. Effect of phenolic compounds and indoleacetic acid on adventitious root initiation in cutting of *Phaseolus aureus*, *Acer saccharinum* and *Acer griseum* [J]. *Horticultural Science*, **18**: 352—354

Lerouge P, Roche P, Faucher C, *et al*, 1990. Symbiotic host-specificity of *Rhizobium meliloti* is determined by a sulphated and acylated glucosamine oligosaccharide [J]. *Nature*, **344**: 781—784

Lian B, Zhou XM, Miransari M, *et al*, 2000. Effects of salicilic acid on the development and root nodulation of soybean seedlings [J]. *J Agronomy & Crop Science*, **185**: 187—192

Lopez-Lara IM, Blok Trip L, Quinto C, *et al*, 1996. NodZ of *Bradyrhizobium* extends the nodulation host range of *Rhizobium* by adding a fucosyl residue to nodulation signals [J]. *Molecular Microbiology*, **21** (2): 397—408

Lopez-Lara IM, Drift KMGM van der, Brussel AAN, *et al*, 1995. Induction of nodule primordia on *Phaseolus* and *Acacia* by lipo-chitin oligosaccharide nodulation signals from broad-host-range *Rhizobium* strain GRH2 [J]. *Plant Molecular Biology*, **29** (3): 465—477

Lopez-Lara IM, Berg JDJ van, Thomas-Oates JE, *et al*, 1995. Structural identification of the lipo-chitin oligosaccharide nodulation signals of *Rhizobium loti* [J]. *Molecular Microbiology*, **15** (4): 627—638

Mariangela H, Gary S, 1997. Molecular signals exchanged between host plants and rhizobia: Basic aspects and potential application in agriculture [J]. *Soil Biol Biochem*, **29** (5&6): 819—830

Malamy J, Carr JP, Klessig DF, *et al*, 1990. Salicylic acid a likely endogenous signal in the resistance response of tobacco to viral infection [J]. *Science*, **250**: 1002—1004

Mergaert P, Vanmontagu M, Holsters M, 1997. Molecular mechanisms of nod factor diversity [J]. *Molecular Microbiology*. 1997, **25** (5): 811—817

Metraux JP, Signer H, Ryals J, *et al*, 1990. Increase in salicylic acid at the onset of systemic acquired resistance in cucumber [J]. *Science*, **250**: 1004—1006

Ovtsyna AO, Geurts R, Bisseling T, *et al*, 1998. Restriction of host range by the sym2 allele of Afghan pea is nonspecific for the type of modification at the reducing terminus of nodulation signals [J]. *Molecular Plant-Microbe Interactions*, **11** (5): 418—422

Paul EA, Clark FE, 1996. Soil Microbiology and Biochemistry [M]. Academic Press Inc. 2ed edition. 215—243

Rasmussen JB, Hammerschmidt R, Zook MN, 1991. Systemic induction of salicylic acid accumulation in cucumber after inoculation with *Pseudomonas*-syringae pv syringae [J]. *Plant Physiol*, **97**: 1342—1347

Roche P, Maillet F, Plazanet C, *et al*, 1996. The common nodABC genes of *Rhizobium meliloti* are host-range determinants [J]. *Proceeding of the National Academy of Sciences of the United States of America*, **93** (26): 15305—15310

Roehrig H, Schmidt J, Walden R, *et al*, 1995. Growth of tobacco protoplast stimulated by synthetic lipo – chitooligosaccharides [J]. *Science* (Washington D C). **269** (5225): 841—843

Saxena AK, Tilak KVBR, 1994. Interaction among beneficial soil microorganisms [M]. *Indian Journal of Microbiology*, **34** (2): 91—106

Schlaman HRM, Gisel AA, Quaedvlieg NEM, *et al*, 1997. Chitin oligosaccharides can induce cortical cell division in roots of *Vicia sativa* when delivered by ballistic microtargeting [J]. *Development* (Cambridge), **124** (23): 4887—4895

Sofie G, 1998. Srchi13, a novel early nodulin from *Sesbania rostrata*, is related to acidic class - chitinases [J]. *The Plant Cell*, **10**: 905—915

Spaink HP, 1996. Regulation of plant morphogenesis by lipo-chitin oligosaccharides [J]. *Critical Reviews in Plant Sciences*, **15**

(5&6): 559—582

Stacey G, Shibuya N, 1997. Chitin recognition in rice and legumes [J]. *Plant & Soil*, **194** (1&2): 161—169

Staehelin C, Granado J, Muller J, *et al*, 1994. Perception of *Rhizobium* nodulation factors by tomato cells and inactivation by root chitinase [J]. *Proceedings of the National Academy of Sciences of the United States of America*, **91** (6): 2196—2200

Stokkermans TJW, Orlando R, Kolli VSK, *et al*, 1996. Biological activities and structures of *Bradyrhizobium elkanii* low abundance lipo chitin-oligosaccharides [J]. *Molecular Plant-Microbe Interactions*, **9** (4): 298—304

Stokkermans TJW, Ikeshita S, Cohn J, *et al*, 1995. Structural requirements of synthetic and natural product lipo-chitin oligosaccharides for induction of nodule primordia on *Glycine soja* [J]. *Plant Physiology*, **108** (4): 1587—1595

Surange S, Kumar N, 1993. Phosphate solubilization under varying pH by *Rhizobium* from tree legumes [J]. *Indian J Exp Biol*, **31**: 855—857

Van Der Drift KMGM, Spaink HP, Bloemberg GV, *et al*, 1996. *Rhzobium leguminosarum* bv. *trifolii* produces lipo-chitin-ligosaccharides with nodE-dependent highly unsaturated fatty acyl moieties: An electrospray ionization and collision-induced dissociation tandem mass spectrometric study [J]. *Journal of Biological Chemistry*, **271** (37): 22563—22569

Voet D, Voet J, 1995. Biochemistry [M]. John Wiley & Sons, Inc. 258—276

Yanni Y, Rizk GRY, Corich Y, *et al*, 1995. Endorhizosphere colonization and growth promotion of indica and japonica rice varieties by *Rhizobium leguminosarum* bv *trifolii*, abstr. O17 [C]. In: Proceedings of the15th Symbiotic Nitrogen Fixation Conference, North Carolina State University, Raleigh, N. C.

Zhang F, Smith DL, 1995. Preincubation of *Bradyrhizobacterium japonicum* with genistein accelerates nodule development of soybean at suboptimal root zone temperatures [J]. *Plant Physiol*, **108**: 961—968

Zhang F, Dashti N, Hynes RK, *et al*, 1996. Plant growth promoting rhizobacteria and soybean nodulation and nitrogen fixation at suboptimal root zone temperatures [J]. *Annal of Botany*, **77**: 453—459

Zhang F, Dashti N, Hynes RK, *et al*, 1997. Plant growth promoting rhizobacteria and soybean growth and physiology at suboptimal root zone temperatures [J]. *Annal of Batany*, **79**: 243—249

Zhou XM, Mackenzie AF, Madramootoo CA, *et al*, 1999. Effects of stem-injected plant growth regulators, with or without sucrose, on grain production, biomass, and photosynthetic activity of field growth corn plants [J]. *J Agron Crop Sci*, **183**: 103—110